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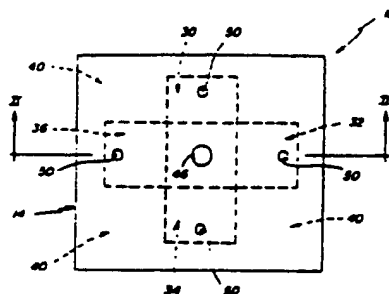
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54 Test device for and method of analysis of a plurality of analytes.

57 A test device and method are disclosed for the clinical analysis of liquids. Test device (10) comprises a plurality of test elements (30-36), each element being adapted to perform a separate analysis. All of the elements are supplied liquid from a single quantity of liquid, and the liquid is conveyed to the test elements through a liquid transport zone. To prevent contamination, control means are incorporated in test device (10) so that the liquid is confined to a plurality of flow paths, each of which extends to only one test element.



TEST DEVICE FOR AND METHOD OF ANALYSIS  
OF A PLURALITY OF ANALYTES

The present invention relates to a device and method useful in the clinical analysis of analytes of a liquid.

5 U.S. Patent Specification No. 3,992,158, discloses a new approach to chemical analysis in which a dry test element contains all the necessary reagents for an extremely accurate quantitative assay on a single drop of test liquid, such as blood  
10 serum. The test element is provided with one or more reagent layers and a spreading layer; the spreading layer is preferably isotropically porous to insure that uniform concentrations of the dissolved or dispersed substances of the test liquid arrive at the  
15 reagent layers for the necessary chemical reactions. The result is a uniform generation of a detectable response predictably related to the concentration of the analyte under study.

The above-described test elements provided a  
20 marked improvement in the state of the art, since they eliminated the plumbing required in apparatus using "wet" techniques and improved the accuracy of the test results. It was the practice to provide a separate test element for each assay, for example one  
25 element for the assay of glucose and another for the assay of blood urea nitrogen (BUN). It was recognized, however, that there would be significant advantages in a test element of this type which could be used for a number of different assays.

30 It is known to provide a single test device for performing a plurality of assays. However, devices developed heretofore have not been generally successful, since reactions from the different assays tend to interfere with each other. One such device  
35 is disclosed in U.S. Patent Specification No.

3,715,192, in which a dip-and-read element is described as having two different reagent layers spaced from each other to define a path through which the test liquid flows. Reagent from one layer is  
5 free to flow into the other layer along the common flow path, and thus, there is potential for cross-contamination. Although a certain amount of cross-contamination can be ignored in qualitative devices, it cannot be ignored when making exact,  
10 quantitative measurements.

In U.S. Patent Specification No. 3,690,836, a multiple test element is disclosed which incorporates a plurality of test devices on a single support. Each of the test devices comprises super-  
15 posed plastic sheets sealed together to define a capillary reaction chamber, one of the sheets having an aperture therein to provide access to the chamber, and an absorbent sheet in the chamber which is adapted to contain a reagent. The disadvantage of  
20 elements of this type is that a separate drop of test liquid must be supplied to each of the test devices; consequently, separate metering operations are required for each assay.

It is an object of the present invention to  
25 overcome the aforementioned problems in the prior art by providing a multi-analyte device for performing a plurality of assays on a single drop of test liquid without interference between the reactions of the respective assays.

30 In accordance with the present invention there is provided a test device for the analysis of a plurality of analytes in a liquid, said device comprising a support member and a cover member joined by an intermediate member, said cover and support mem-  
35 bers having opposed surfaces defining a liquid transport zone in which the surfaces are spaced apart

a distance effective to induce capillary flow of said liquid, one of said members having access means for receiving and transporting a quantity of the liquid into said zone, said zone having therein a reagent composition for the generation of a detectable response indicative of an analyte characterized in that a plurality of individual test elements are disposed in said zone on one of said members at locations spaced from each other and from said access means, one of said elements comprising said reagent composition and another of said elements comprising a reagent composition for the generation of a detectable response indicative of another analyte, and control means for confining liquid flow to a plurality of predetermined flow paths each of which extends from said access means to only one of said test elements whereby the analytes in said quantity of liquid are detectable by the respective test elements without cross-contamination between the test elements.

In accordance with the present invention there is also provided a method of analyzing for a plurality of analytes in a liquid comprising the steps of depositing a quantity of the liquid into a receiving zone of a test device characterized by subjecting the liquid to capillary flow from the receiving zone along a plurality of separate flow paths in said device, each path extending to a single test element capable of reacting with a particular analyte to provide a detectable response whereby liquid is directed along each of said paths without cross-contamination between said test elements, and detecting said response at each of said test elements.

Embodiments of the present invention will

now be described, by way of example, with reference to the accompanying drawings in which:

Fig. 1 is a plan view of a test device in accordance with the invention;

5 Fig. 2 is a sectional view taken generally along the plane of the line II-II in Fig. 1;

Fig. 3 is a plan view similar to that of Fig. 1, but illustrating the liquid flow paths to each test element;

10 Fig. 4 is a fragmentary sectional view similar to that of Fig. 2, but illustrating an alternative embodiment;

Fig. 5 is a sectional view, similar to that of Fig. 2, illustrating yet another embodiment;

15 Fig. 6 is a plan view of still another embodiment;

Fig. 7 is a sectional view taken generally along the plane of the line VII-VII of Fig. 6; and

20 Fig. 8 is a plan view similar to that of Fig. 6, but illustrating the controlled liquid flow paths provided to each test element.

The device and the method of this invention are described, in connection with certain preferred embodiments, as a device and a method for analyzing  
25 blood serum. In addition, the invention can be applied in the radiometric detection of analytes of other liquids, for example, industrial liquids.

European Patent Application No. 79302338.3, published September 3, 1980, entitled "Liquid  
30 Transport Device, Method of Transporting A Liquid, Method of Analyzing A Liquid, and Test Element for Use in Analysis of a Liquid," describes generically the use of opposed transport surfaces to distribute a liquid drop by capillary action to a plurality of  
35 test areas. The present invention is an improvement on the invention disclosed in Application No. 79302338.3.

In Figs. 1 and 2, there is shown a device 10 constructed in accordance with one embodiment of the invention. Device 10 comprises a support member 12, preferably transparent, and a cover member 14 which have exterior surfaces 16 and 18, respectively. Members 12 and 14 are sealed at their peripheries to an intermediate member 20 (Fig. 2), the sealing being effected for example by an appropriate adhesive or by ultrasonic welding. It will be apparent that members 12, 14 and 20 could be integrally formed by conventional techniques. Except for apertures 46, 50, discussed hereinafter, the space enclosed by members 12, 14 and 20 is preferably airtight.

At the centre of the interior surface of support member 12 is disposed a boss 22 which, together with the interior wall 24 of member 20, defines a plurality of cavities extending radially from the boss. These cavities are filled with a plurality of individual test elements 30, 32, 34 and 36 (Fig. 1) each of which contains all the reagents necessary for the detection of a different one of the several analytes expected to be present in the test liquid. Intermediate member 20 includes a spacer 40 (Fig. 1) at each of the corners of device 10 to fill portions of the cavities not filled by the test elements.

The top surfaces of test elements 30, 32, 34 and 36, as well as that of the boss 22, are spaced a distance "s" from an interior surface 42 of cover member 14 (Fig. 2). The distance "s" is effective to induce capillary flow of liquid introduced between the cover member 14 and either the boss 22 or the test elements, and to thus create a liquid transport zone 38. Zone 38 is bounded in the vertical direction, as viewed in Fig. 2, by wall 24 and spacers 40 of intermediate member 20. Preferably "s" is between

about 25 microns and about 500 microns. To permit the introduction of the test liquid, an access aperture 46 is formed in cover member 14, which functions as a receiving zone. Alternatively, the  
5 access aperture can be provided in the centre portion of member 12 through boss 22. The size and shape of the access aperture 46 is such as to accommodate a drop of test liquid while at the same time ensuring that the drop will contact both surface 42 and the boss  
10 22. Because of the capillary spacing "s," such contact immediately causes the drop to spread under the influence of capillary attraction. Although the shape of the wall defining access aperture 46 is not critical, and can be cylindrical as shown, cross-  
15 sectional shapes such as a regular hexagon (not shown) provide interior corners that act to centre the drop within the aperture. To prevent cross-contamination of the reagents from the test elements, aperture 46 is not located immediately adjacent any  
20 test element.

For a 10- $\mu$ l sized drop, a convenient size of such a hexagonally shaped access aperture is one in which the outside diameter is about 0.26cm. The corners of the hexagon should be as sharply defined as  
25 possible, and in no case have a radius of curvature greater than about 0.4mm.

As the liquid advances, as per arrows 48 (Fig. 2), between surface 42 and the top surfaces of boss 22 and the test elements, the trapped air must  
30 exit. To provide a vent means for the trapped air, vent apertures 50 are formed in cover member 14 almost at the edge of zone 38 as defined by intermediate member 20. Each test element 30, 32, 34 and 36 has a vent aperture 50 disposed above it.

35 Liquid flow in zone 38 can occur only in the direction in which the trapped air is being expelled. Thus, in accordance with one aspect of the

invention, a control means, which includes apertures 50 and intermediate member 20, confines the liquid to flow along a plurality of predetermined controlled flow paths (Fig. 3) such that each of the test elements 30, 32, 34 and 36 is operatively disposed in alignment with only one of those paths. The paths are identified in Fig. 3 as 52<sup>I</sup>, 52<sup>II</sup>, 52<sup>III</sup>, and 52<sup>IV</sup>, respectively, leading from aperture 46 to the vent aperture 50 associated with a particular test element. It is this construction that prevents significant contamination of one test element by a reagent of another test element, inasmuch as flow proceeds to test elements 30, 32, 34 and 36, rather than between them.

In accordance with another aspect of the invention, each of the test elements 30, 32, 34 and 36 comprises one or more layers that are more or less absorbent of the liquid under test. Such absorbency further prevents cross-flow between test elements which would create contamination. That is, as the sample liquid is absorbed, connective paths are removed between test elements that might otherwise remain after overall flow has ceased. Diffusion through quiescent liquid in the zone 38 is thus prevented.

Preferably each test element 30, 32, 34, and 36 comprises one or more reagent layers (Fig. 2) having a variety of binder compositions. For example, gelatin, cellulose acetate butyrate, polyvinyl alcohol, agarose and the like can be used, the degree of hydrophilicity of a layer 54 depending upon the material selected.

Additional layers, such as a layer 56, can be disposed above layer 54 to provide a variety of chemistries or functions. For example, these can provide, either in layer 56 alone or together with



layer 54, a reagent composition. Filtering, registration and mordanting functions can be provided also by such additional layers, as described in U.S. Patent Specification No. 4,042,335. Thus, layer 56  
5 can comprise a reagent, such as an enzyme, and a binder.

As used herein, "reagent" in "reagent composition" means a material that is capable of interaction with an analyte, a precursor of an analyte, a  
10 decomposition product of an analyte, or an intermediate. Thus, one of the reagents can be a preformed, radiometrically detectable species that is caused by the analyte of choice to move out of a radiometrically opaque portion or layer of the element, such as  
15 layer 56, into a radiometrically transparent portion or layer, such as a registration layer which can be layer 54.

The noted interaction between the reagents of the reagent composition and the analyte is therefore meant to refer to chemical reaction, catalytic  
20 activity as in the formation of an enzyme-substrate complex, or any other form of chemical or physical interaction, including physical displacement, that can produce ultimately a detectable response in the  
25 test element. The assay of the element is designed to produce a response signal that is predictably related to the amount of analyte that is present.

The preferred device is designed for a radiometric detection of the response, that is, by  
30 impinging electromagnetic energy on the test elements. The response is then measured, preferably radiometrically. As is well known, radiometric detection includes both colorimetric and fluorimetric detection, depending upon the indicator reagent  
35 selected for the assay.

Alternatively, the device of the invention

can be used with test elements having any kind of detectable response, the device being suitably modified, if necessary, to permit such alternate form of detection.

5           Each of the test elements 30, 32, 34 and 36  
can test for a different analyte. Preferably, the  
assays are all oxygen-independent, as the flow of  
blood or blood serum into zone 38 tends to seal off  
the elements from any additional oxygen. Typical  
10 analytes which can be tested include BUN, total  
protein, bilirubin and the like. The necessary  
reagents and binder or vehicle compositions for, e.g.  
layers 54 and 56 of the test elements for BUN, total  
protein, and bilirubin, can be those described,  
15 respectively, in U.S. Patent Specifications Nos.  
4,066,403; 4,132,528; and 4,069,016 or 4,069,017.  
Thus, test element 30 can be designed to test for  
BUN, element 32 to test for total protein, element 34  
to test for bilirubin, and element 36 to test for a  
20 fourth analyte. Alternatively, element 36 can be a  
duplicate of any of elements 30, 32 or 34 to provide  
a confirming reading, or it can be a blank to permit  
standardization of the analyzer.

As is apparent from the preceding, the quan-  
25 tity of liquid preferably is added to aperture 46 and  
zone 38 in the form of a drop. Alternatively, other  
forms of liquid introduction can be used. For exam-  
ple, a pipette tip could be inserted into aperture 46  
to dispense the desired quantity of liquid into zone  
30 38.

Quantitative detection of the response  
produced in each test element by the analyte is  
preferably made, after a suitable development period,  
by scanning the device 10 through member 12 with a  
35 photometer or fluorimeter. Either a reflective or a  
transmissive mode can be used, depending upon the

presence or absence of an opacifier in layer 56. A variety of such instruments can be used, for example the radiometer disclosed in U.S. Patent Specification No. 4,152,390, or the photometer described in U.S.

5 Patent Specification No. 4,119,381. Of course, such instruments would be adapted to separately read each test element of device 10, such as through the use of filters and means to index the scanning beam to each test element. After detection, the device 10 is  
10 discarded.

Preferred materials for members 12, 14 and 20 are non-fibrous plastics materials that are substantially impervious to aqueous liquids. Examples include acetates, polystyrene, polyethylene, ABS plastic and  
15 polycarbonate.

Any other radial design can be used, besides that of Fig. 1, to provide, for example, 3, 5, 6 or n-test elements that are preferably equidistant from access aperture 46.

20 As an additional alternative, test elements 30, 32, 34 and 36 can be located on cover member 14 instead of member 12, or on both of said members, in suitably formed cavities therein.

The embodiment shown in Fig. 4 is the same  
25 as the embodiment shown in Figs. 1-3, except that the boss 22 has been omitted from the embodiment of Fig. 4. In such an arrangement, the distance "s" must still be of a magnitude effective to induce capillary flow of liquid; it should be noted that surface 42  
30 and layer 56 of element 32 are still spaced from each other, but by a distance "s'" which is less than distance "s." In this embodiment physical separation of the elements 30, 32, 34 and 36 aids in preventing cross-contamination.

35 The embodiment of Fig. 5 is a preferred form of the embodiment illustrated in Figs. 1-3. In some

instances, the initial flow of liquid over boss 22 proceeds to only one or two of the test elements 30, 32, 34 and 36 due to the non-directional nature of the surface of boss 22. That is, a smooth, 5 non-directional boss surface could result in exclusive flow to only one side, and one test element, of the test device. If such is the case, device 10a should be used to alleviate the problem. Parts in device 10a similar to those previously 10 described bear the same reference numeral to which the distinguishing suffix "a" is added.

Device 10a is identical to the device of Fig. 2 in that a plurality of test elements, of which only elements 36a and 32a are shown, are disposed 15 about boss 22a; boss 22a and the test elements all have exterior surfaces spaced a capillary distance from surface 42a of cover member 14a. Cover 14a includes an aperture 46a. To ensure that incoming liquid first completely wets all of the surface of 20 boss 22a, and then proceeds to all of the test elements, the following modifications are provided. Surface 57 of boss 22a is raised above the exterior surface of layers 56a. In addition, surface 42a is recessed slightly at portions 58 which are disposed 25 directly opposite to elements 32a, 36a, etc. However, distance "s'" between the recessed portions of surface 42a and the exterior surface of layer 56a is still a capillary spacing, although larger than the spacing existing over boss 22a. This embodiment is 30 effective because the first portion of the liquid that enters zone 38a "sees" a temporary energy barrier at the edges of surface 57 and the edges 59 in surface 42a created by the portions 58. The meniscus prefers to fill the zone directly above boss surface 35 57, rather than proceed into the wider spacing denoted as "s'." After the zone directly above

surface 57 is filled, however, the pressure of the incoming liquid overcomes the temporary energy barrier, and the liquid proceeds to fill the zone above all the test elements.

5           The interior surface 42 of the cover member 14 need not be smooth, as shown in Fig. 2. In accordance with yet another aspect of the invention, control of the liquid flow paths can also be obtained by portions of one or both opposed surfaces having a  
10           plurality of exposed grooves. Such an embodiment is illustrated in Figs. 6-8, wherein parts similar to those previously described bear the same reference numeral to which the distinguishing suffix "b" is appended. Thus, a device 10b comprises a support  
15           member 12b, a cover member 14b and an intermediate member 20b that secures the two together as described previously. In this embodiment, test elements 30b, 32b, 34b and 36b are recessed into appropriately-shaped cavities of support member 12b. A vent  
20           aperture 50b is located at an end of each test element opposite to the element end adjacent access aperture 46b.

          Unlike the previously described embodiment, the test elements are not disposed equidistant from  
25           access aperture 46b, but rather are all to one side thereof. In such an arrangement, a flow path 60 (Fig. 6) from aperture 46b to, say, aperture 50b for element 30b, would be in fluid contact with more than one test element. Such a path would likely contaminate element 30b with reagents from element 32b.  
30           Therefore, the controlled flow of the liquid must proceed indirectly to each test element, in the manner depicted by arrows 52b<sup>I</sup>, 52b<sup>II</sup>, 52b<sup>III</sup>, and 52b<sup>IV</sup> (Fig. 8). Flow between elements 30b,  
35           32b, etc., is to be avoided to prevent cross-contamination.

A preferred construction to provide such controlled flow is one in which the interior surface of member 14b is grooved (Fig. 7). Such grooves 70 are preferably parallel and linear, extending generally perpendicularly from a plane of intersection passing through aperture 46b and line 69 (Fig. 6) to the row of apertures 50b delineated by line 71. In turn, the exposed surface portions of member 12b that oppose grooves 70 are also grooved at 72, but at an angle to grooves 70. Preferably, that angle is about 90 degrees.

The grooves 70 and 72 can have a variety of shapes, including sawtooth, truncated and sinusoidal shapes. The pitch of the grooves can be constant or variable. The groove dimensions can also be varied, so long as capillary flow is maintained between the surfaces. A representative set of grooves for use with blood serum comprises a set of sawtooth grooves in which the width of a groove, and of the ridge that forms it, is in each case about 13 microns, and the depth of the grooves is about 7 microns.

To prevent contamination between elements, preferably no grooves 72 are included in those portions 80 of the member 12b that lie between test elements. If further protection of each test element is desired, a separation wall, not shown, can be included along portions 80.

The two sets of grooves angled with respect to each other control the flow pattern of the liquid as it advances from aperture 46b. More specifically, the advancing edges of the liquid appear to take on the shape of the edges of the grooves across which the liquid is moving. The groove edges in this embodiment extend rectilinearly. Thus the liquid appears to expand as a rectangle away from aperture 46b within zone 38b, until it reaches member 20b; it

continues to move within grooves 72 towards the more distant test elements 30b and 36b. At the same time, grooves 70 carry the liquid flow to the entrance portions of the test elements. The net effect is an  
5 apparent flow that follows the paths 52b<sup>I</sup> etc. (Fig. 8), so that the liquid turns corners as necessary to provide flow to the test elements without flowing in significant amounts from one test element to another.

10 Because of the additional flow control provided by grooves 70 and 72, the embodiment of Figs. 6-8 need not provide a liquid transport zone that is as airtight as the zones of the previous embodiments.

15 Yet another alternative is to provide, as the air vent means, one or more slots 100 in intermediate member 20b, shown in phantom in Figs. 7 and 8, in place of air vents 50b. If one continuous slot 100 is used (Fig. 8), then the portion adjacent each  
20 of the test elements constitutes the air vent for that particular test element.

Still another alternative (not shown) is to form the access aperture in member 20b adjacent grooves 72, rather than in cover member 14b.

25 It will be readily appreciated that an additional series of test elements (not shown) can be arranged in a row on the opposite side of aperture 46b, to create a device having an axis of symmetry along line 69.

CLAIMS

1. A test device for the analysis of a plurality of analytes in a liquid, said device comprising a support member and a cover member joined by an intermediate member, said cover and support members having opposed surfaces defining a liquid transport zone in which the surfaces are spaced apart a distance effective to induce capillary flow of said liquid, one of said members having access means for receiving and transporting a quantity of the liquid into said zone, said zone having therein a reagent composition for the generation of a detectable response indicative of an analyte characterized in that a plurality of individual test elements (30-36; 30a-36a; 30b-36b) are disposed in said zone (38; 38a; 38b) on one of said members (12, 14, 20; 12a, 14a, 20a; 12b, 14b, 20b) at locations spaced from each other and from said access means (46; 46a; 46b), one of said elements comprising said reagent composition and another of said elements comprising a reagent composition for the generation of a detectable response indicative of another analyte, and control means (20, 50; 20a, 50a; 50b, 70, 72) for confining liquid flow to a plurality of predetermined flow paths (52<sup>I</sup>-52<sup>IV</sup>; 52b<sup>I</sup>-52b<sup>IV</sup>) each of which extends from said access means (46; 46a; 46b) to only one of said test elements whereby the analytes in said quantity of liquid are detectable by the respective test elements without cross-contamination between the test elements.

2. A device according to Claim 1, characterized in that said control means includes a vent means (50; 50a; 50b) associated with each of said test elements for venting air from said zone ahead of liquid flowing to the test element.



3. A device according to Claim 1 or 2, characterized in that said test elements each comprise an absorbent layer (54, 56; 54a, 56a; 54b, 56b) containing at least one reagent.

5 4. A device according to Claim 1, 2 or 3, characterized in that each of said test elements includes all the reagents necessary for a radiometric assay of a particular analyte.

10 5. A device according to Claim 3, characterized in that said one element includes the reagents necessary for the detection of total protein and said other element includes the reagents necessary for the detection of blood urea nitrogen.

15 6. A device according to any one of Claims 1 to 5, characterized in that said test elements are equidistant from said access means.

7. A device according to any one of Claims 1 to 5, characterized in that said test elements are arranged in a side-by-side relationship.

20 8. A device according to any one of Claims 1 to 5 and 7, characterized in that said control means includes a plurality of exposed grooves (70, 72) in at least one of said surfaces.

25 9. A device according to Claim 8, characterized in that said grooves (70, 72) are substantially linear and parallel and are orientated to confine the flow of liquid to said predetermined flow paths.

30 10. A device according to Claim 9, characterized in that both of said surfaces include a plurality of exposed grooves (70, 72), the grooves of one surface being orientated at a 90 degree angle with respect to the grooves of the other member.

35 11. A device according to any one of the preceding Claims characterized in that one of said members (12, 14, 20; 12a, 14a, 20a; 12b, 14b, 20b) is transparent.

12. A method of analyzing for a plurality of analytes in a liquid comprising the steps of depositing a quantity of the liquid into a receiving zone of a test device characterized by subjecting  
5 the liquid to capillary flow from the receiving zone along a plurality of separate flow paths in said device, each path extending to a single test element capable of reacting with a particular analyte to provide a detectable response whereby liquid is  
10 directed along each of said paths without cross-contamination between said test elements, and detecting said response at each of said test elements.

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FIG. 1

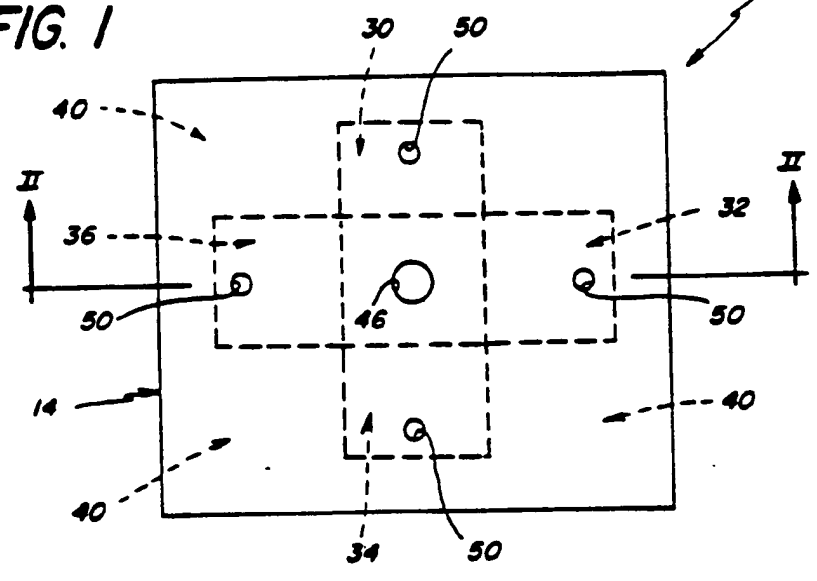


FIG. 2

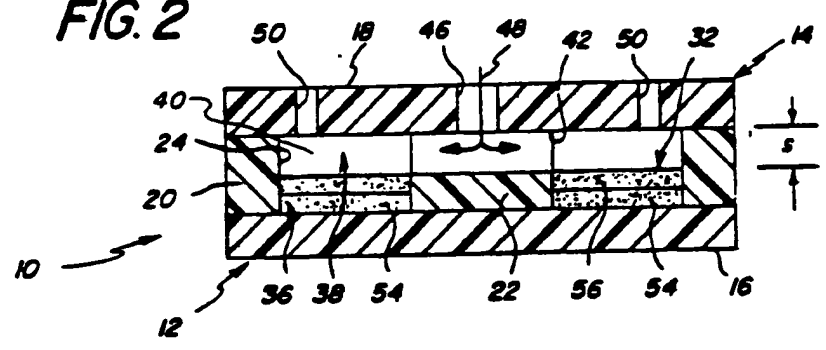
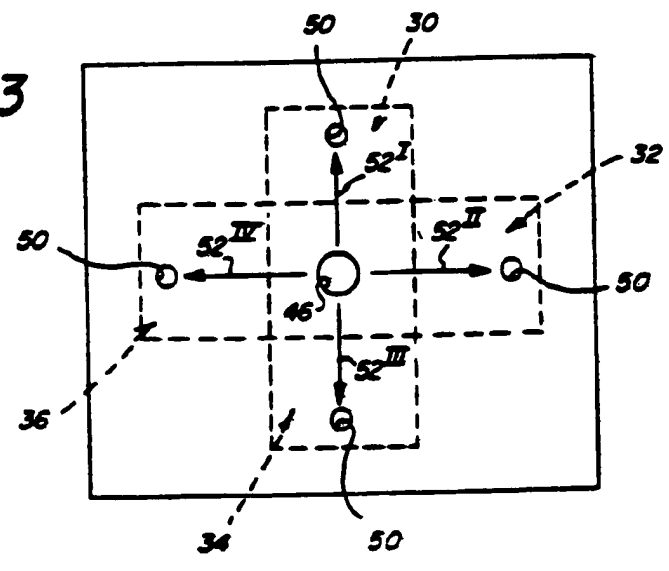


FIG. 3





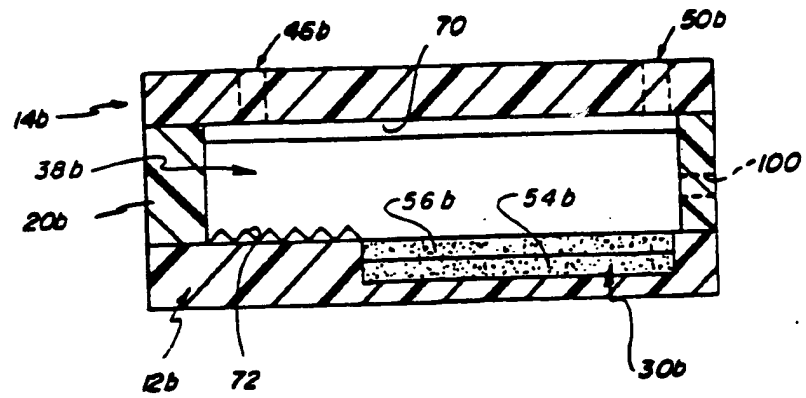


FIG. 7

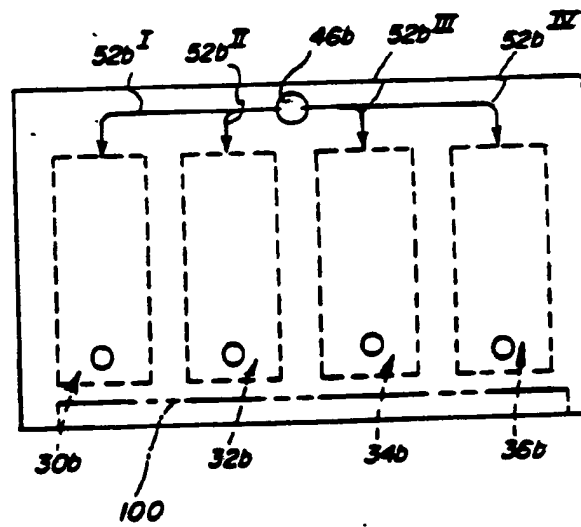


FIG. 8





European Patent  
Office

EUROPEAN SEARCH REPORT

0034049  
Application number  
EP 81 30 0494

-2-

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. CL.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
P	<u>DE - A - 2 934 760</u> (FUJI PHOTO FILM)  * From page 1, line 1 to page 2, line 4; figures 1-3 *  -----	1,3,6	
P	<u>DE - A - 2 949 747</u> (FUJI PHOTO FILM)  * From page 16, line 28 to page 17, line 8; figure 4 *  -----	1,3,6	
			TECHNICAL FIELDS SEARCHED (Int. CL.)